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INFLUENCE OF HEAT STRESS ON GROWTH PERFORMANCE AND SOME BLOOD CONSTITUENTS OF *OREOCHROMIS NILOTICUS* FED ASCORBIC ACID

(With 2 Tables and 6 Figures)

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تأثير الإجهاد الحرارى على النمو وبعض مكونات الدم فى أسماك البلطى النيلى المغذاه على حامض الأسكوربيك

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كان الهدف من هذه الدراسة هو اختبار تأثير الإجهاد الحرارى (٣٣ م°) منفردا أو مع إضافة حامض الأسكوربيك (٣جم /كجم مادة جافة) على السلوك والنمو ومعدل النفوق وكذلك بعض مكونات الدم فى البلطى النيلى. قسمت ١٢٠ سمكة إلى ثلاثة مجموعات طبقا للوزن والطول: المجموعة (A) ضابطة (لم تعرض إلى إجهاد حرارى ولم تغذى على حامض الأسكوربيك), المجموعة الثانية (B) عرضت إلى درجة حرارة مياه مرتفعة (٣٣ م°) فى حين تعرضت المجموعة الثالثة (C) لنفس درجة الحرارة (٣٣ م°) مع التغذية على حامض الأسكوربيك بواقع ٣ جرام/ كجم مادة جافة. ولقد تم تسجيل التغيرات فى السلوك ,التغذية والنفوق يوميا وكذلك طول ووزن الجسم على أساس فردى أسبوعيا. تم جمع عينات الدم كل أسبوعين خلال الفترة التجريبية آلتي أستمرت ست أسابيع. وفى نهاية التجربة تم ذبح كل الأسماك ونزع كل من الكبد, الطحال والغدد الجنسية ووزنها فى الحال. وأوضحت النتائج أن تعريض الأسماك لدرجة حرارة مياه عالية قد أظهرت سلوكا غير طبيعى كما أدت إلى ارتفاع فى معدل النفوق وانخفاض فى وزن وطول الجسم. بالإضافة إلى النسبة بين الغدد ووزن الجسم مثل نسبة الكبد, الطحال والغدد الجنسية (المبيضين والخصيتين). كذلك أدى الإجهاد الحرارى إلى زيادة تركيز الهيموجلوبين والمكونات الخلوية بالدم. ولقد تضاعف تركيز الجلوكوز فى السيرم فى المجموعة المعرضة للإجهاد الحرارى (B) مقارنة بالكنترول (A). ولقد لوحظ أن تركيز البروتين الكلى فى السيرم يقل فى المجموعة (B) بالمقارنة بالمجموعة (A) ويرجع إلى انخفاض تركيز الجلوبيولين بدرجة أكبر من الألبيومين. ولقد أوضحت النتائج أن الإجهاد الحرارى قد أدى إلى زيادة متوسط قيم أنزيمات AST & ALT بمعدل ٥٨ & ٢٨ % على التوالى. كما أدى إلى انخفاض فى تركيز أنزيم الفوسفاتيز القاعدى فى

السيرم. هذا وقد أمكن التغلب على التأثيرات السالبة للإجهاد الحرارى على البلطى النيلى بإضافة حامض الأسكوربيك فى العليقة حيث أدت إضافته إلى الحفاظ على المستوى الطبيعى للسلوك, النمو, معدل النفوق ونسبة الغدد إلى وزن الجسم فى الأسماك المعرضة للإجهاد الحرارى. ومن الملاحظ أن حامض الأسكوربيك كان مفيدا فى التغلب على التأثيرات الضارة للحرارة المرتفعة على مكونات السيرم الأيضية والأنزيمات. مما سبق يمكن أن نخلص إلى أن إضافة حمض الأسكوربيك إلى عليقة المكعبات للأسماك (٣جرام / كجم مادة جافة) قد يكون مفيدا للتغلب على التأثيرات الضارة لحرارة المياه المرتفعة على البلطى النيلى فى نظم الاستزراع السمكى المكثف فى المزارع المغلقة.

SUMMARY

The objective of this experiment was to examine the effects of heat stress (33 °C) or heat stress with ascorbic acid (AA) supplementation [3g AA/kg feed on dry matter (DM) basis] on behavior, growth performance, mortality rate and some blood constituents in *Oreochromis niloticus*. A total number of 120 fish were alloted according to body weight and length into three groups. The first group (A) served as control. The second group (B) exposed to high water temperature (33 °C), while the third group (C) exposed to high water temperature (33 °C) and supplemented with dietary ascorbic acid, 3g/kg DM. Daily changes in their behavior, feeding pattern and mortality rate as well as weekly body weight and body length were recorded on individual basis. Blood samples were taken biweekly. At the end of the experiment all fish were slaughtered and the liver, spleen and gonads were weighed. The experimental period lasted for six weeks. Fish which were exposed to high water temperature exhibited abnormal behavior and higher mortality rate as well as lower body weight, body length and relative body weights, i.e. HSI, SSI, fGSI and mGSI. Also, heat stress increased ($P<0.01$) hemoglobin and hematocrit values. Serum glucose concentration in group B was twice as much as in group A. Serum total protein concentration was lower in group B than in group A due to the decrease in serum globulin rather than albumin. Heat stress increased ($P<0.05$) the overall means of serum AST and ALT concentrations by about 58 and 28%, respectively, while it decreased the concentration of serum alkaline phosphatase. Such negative effects of heat stress on *O. niloticus* raised in closed system could be counteracted by dietary ascorbic acid at 3g/kg DM, since its supplementation maintained normal behavior, body growth, mortality rate and body relative weights in fish exposed to heat stress. Also, ascorbic acid is most beneficial in

counteracting the adverse effects of high water temperature on serum metabolites and enzymes. It was concluded that addition of dietary ascorbic acid (3g/kg DM) may be useful to counteract the adverse effects of high water temperature on Oreochromis niloticus in intensive closed system.

Key Words: Fish, ascorbic acid, heat stress. blood

INTRODUCTION

The water quality including high water temperature is the main problem facing fish farming, particularly in intensive closed system (Krom et. al. 1985 a and b; Forsberg and Summerfelt 1992 and Abdel Hady et al., 1993).

Qualitative and quantitative changes in composition of water streams were found to be influenced by fluctuations in its thermal characteristic (Shehata, 1990 and Mason, 1991). High water temperature (33 °C) during mid-summer (June – September) in the middle east area leads to mass fish mortality (Darley, 1982 and krom et. al. 1985 a and b). The temperature above 30 °C is stressful to most fishes (Kilambi and Robinson, 1979 and Barila and Stauffer, 1980). Moreover, the fish mortality occurred in mid – summer may be due to insufficient oxygen level in this period (Krom et.al.1985 a and b). Drainge using water for cooling by power generating stations and drainage this hot water to natural water streams stimulated studies on thermal effects on fish production (Mason, 1991). Shehata, (1990) reported that drainage water used for cooling by the large generating power station in Assiut (South Egypt) through the river Nile elevated the water temperature in wide area of the river to 33-35°C. Studies on water temperature on performance of *Oreochromis niloticus* are limited. Furthermore, studies on effects of antiheatstress such as ascorbic acid on behavior and response of fish are also limited. Since the ability of fish to synthesize ascorbic acid is limited, and they have to depend upon the dietary sources (Lovell, 1984 and Hardie et. al., 1991). Meyer (1974) and Teskeredzic, et. al., (1989) attributed the high mortality rate of commercial catfish and salmon to the low body stored ascorbic acid. In Upper Egypt, the water temperature increases over 30 °C in summer, particularly in intensive closed system which leads to mass fish mortality. The objectives of this study are, to determine the effects of

high water temperature on growth performance, mortality rate and some blood constituents and to determine the effects of ascorbic acid supplementation on growth performance, mortality rate and some blood constituents of *Orechromis niloticus* exposed to high water temperature under Upper Egypt conditions.

MATERIAL and METHODS

One hundred twenty healthy fish, average body weight of 76.22 ± 0.29 g and average body length of 16.13 ± 0.05 cm., were used in the present experiment which lasted 42 days. Fish were kept in large metallic (800-L) aquaria, acclimatized to laboratory conditions for two weeks and divided randomly into three separate groups of 40 fish each. The temperature of water in the first aquarium averaged 26.09 ± 0.25 °C and was considered as a control, while water temperature in the other two aquaria was raised and maintained at 33°C by using thermostatically regulated water heaters. This degree (33 °C) was chosen since it had the optimum harmful effect and led to high mortality rate of the fish (Krom et. al. 1985 a). In addition, Shehata (1990) reported that the Upper incipient lethal temperature (UILT) of O. niloticus is 34 °C. The first group (A) served as non-supplemented ascorbic acid- non-heat exposed, control. The second group (B) exposed to high water temperature (33 ° C), while the third group (C) exposed to high water temperature and fed ascorbic acid (3g/kg DM). This dose of ascorbic acid was used since it was found to yield the highest growth rate of tilapia in intensive culture (Anadu et. al. 1990 and Hussein, 1995), enhance significantly antibodies production and complement activity in the fish (Li and Lovell, 1985) and thus it increases the resistance for different physical or environmental stresses or contaminants (Halver, 1985 and Hussein, 1995). Fish were fed twice daily a pelleted ration at a rate of 3% of body weight. The pellets were coated with carboxy methylcellulose to minimize leaching. The rations used in feeding of group C were supplemented with 3g ascorbic acid/kg feed. The pellets were prepared weekly and were stored at 10 °C till feeding to avoid any deterioration effects (Waagbø et.al.,1989). In all the aquaria, continuous aeration was maintained throughout the experimental period. The experimental aquaria were cleaned regularly at 3-day intervals. In case of group B and C, the aquaria were refilled with heated water to maintain fish at constant experimental water temperature (33 °C). Body weight and length were weekly recorded on individual basis and feed was readjusted.

The condition factor (K value) was estimated according to Oni et. al. (1983) using the following formula, K value = Body weight x 100/ (body length)^3. Changes in behavior, feeding pattern and mortality, if any, were recorded regularly

Individual blood samples of five fish per group were taken biweekly by puncture of the caudal peduncle vessels to determine some blood constituents. Adequate amounts of whole blood in heparinized small plastic vials were used for determination of hemoglobin (Hb) and hematocrit (PCV%) by using kits (Diamond Diagnostics, Egypt) and capillary method, respectively. Blood samples were collected, transferred to centrifuge tubes and allowed to clot at room temperature (22-24 °C). Serum was separated by centrifugation at 3000 rpm for 20 min. and stored in glass vials at – 20 °C until biochemical analyses. Serum concentrations of glucose (mg/dl), total cholesterol (mg/dl) and triglycerides (mg/dl) were determined colorimeterically using commercial test kits (Biocon, Germany). Serum concentrations of total protein (g/dl) and albumin (g/dl) were determined using kits (Diamond Diagnostics, Egypt). Serum globulin concentration was calculated by difference between total protein and albumin. Serum aspartic aminotransferase (AST,u/l) and alanine aminotransferase (ALT, u/l) concentrations were determined by using kits (Diamond Diagnostics, Egypt). Serum concentrations of alkaline phosphatase (IU/L) was determined by using test kits (Stanbio, USA).At the end of the experimental period all fish were weighed, slaughtered and liver, spleen and gonads were weighed immediately. The liver (HSI), spleen (SSI) and gonad (GSI) indices were calculated according to the following formulas: HSI = liver weight x 100 / gutted fish weight (Jangaard et. al. 1967), SSI = spleen weight x 100 / fish weight (Rosenthal et. al.1984) and GSI = gonad weight x 100 / fish weight (Tseng and Chan 1982).

The obtained data were statistically analyzed using the GLM procedures of SAS (1987) for personal computer. Treatment effects were also examined by one-way ANOVA (Steel and Torrie, 1980).

RESULTS and DISCUSSION

1- Fish behavior

The fish which were exposed to 33 °C water temperature (group B) exhibited remarkable signs of distress, including fast swimming, erratic movement, great surfacing frequency to gulp atmospheric air, loss of body equilibrium, i.e turning the body upside down, and decreased

attention to the feeding. In contrast, fish maintained at the same water temperature but supplemented with dietary ascorbic acid (3g/kg DM, group C) did not show much deviation from the normal behavior of control (group A). Such response to heat stress may be attributed to the decrease in oxygen concentration due to high water temperature and/or to the increased respiration rate and heart rate (Dheer, 1988 and Mason, 1991). The normal behavior of group C (AA-supplementation) may be due to the role of ascorbic acid which inhibits some enzymes involved in the biosynthesis of corticosterone (Brake, 1989).

2- Growth performance

Body weight gain and body length increment were negatively affected by heat stress, group B versus group A. Such adverse effect of heat stress was found to be more pronounced at the second and fourth weeks of the experimental period. On the other hand, body weight gain and length increment did not significantly differ ($P>0.05$) between fish in control group (A) and heat stress- ascorbic acid-treated group (C, Table 1 and Fig.1). Otherwise, there were no significant differences in condition factor (K value) among fish groups. Similar results were obtained by Hussein (1995) who showed that K value did not affect by the stressors. The negative effect of heat stress on growth performance might be attributed to the reduced feed intake due to high water temperature (Li and Lovell, 1985 and Mason, 1991) and the increased energy required for high respiration rate (Huges et. al, 1983 and Armstrong, 1998). Hughes et. al., (1983) reported that the increased respiration rate during heat stress may be related to high water temperature as well as to the decrease of its oxygen content. Korwin-Kossokowski and Jezierska, (1985) found that ascorbic acid was effective in counteracting the adverse effects of high water temperature on growth performance, since it increases oxygen consumption and the efficiency of its binding with hemoglobin. In addition ascorbic acid was found to increase thyroid hormones (Fenster, 1987 and Hussein, 1995) due to the increase of thyroid iodine uptake (Abdel Wahab et.al, 1975). Thus, both of high oxygen uptake and thyroid hormones concentrations stimulate metabolism and/or growth.

3- MORTALITY RATE

The highest ($P<0.01$) mortality rate was recorded in group B, which was exposed to high water temperature, while no significant difference ($P>0.05$) between control group (A) and that exposed to the high water temperature (33 °C) and supplemented with ascorbic acid (group C, Fig.2). The increased mortality rate in heat stressed - group

might be attributed to the decrease in body weight (Table 1) due to loss of appetite (Krom et. al., 1985 b). In addition Robertson et. al (1987) proved that heat stress evoked hypersecretion of cortisol. An increase of cortisol hormone is generally associated with downregulation of the immune system (Ellsaesser and Clem, 1987). Reduction of body weight and downregulation of immune system increased susceptibility to disease which may have led to death. However, the mortality rate is decreased in group C, exposed to the same temperature but supplemented with ascorbic acid. This result might be attributed to the role of ascorbic acid which improved body gain (Table1). Moreover, Brake, (1989) found that ascorbic acid is essential to maintain normal immune processes during physiological stress, since it inhibited the enzymes responsible for corticosterone biosynthesis which consequently lead to immuno-suppression.

4- Body relative weights

Fish group exposed to heat stress (B) tended to have lower hepatosomatic (HSI), spleensomatic (SSI), female gonadosomatic (fGSI) and male gonadosomatic (mGSI) indices than those of controls (A). Similarly, group C supplemented with ascorbic acid tended to have the same trend, with exception of SSI which was relatively higher than that of controls (Table 1 and Fig.3) .The increased SSI of ascorbic acid supplemented group may be related to the role of ascorbic acid in reducing the concentration of plasma corticosterone. This is in accordance with the findings of Waagbø et. al. (1989). Halver, (1985) reported that the high level of corticosterone led to a prominent decrease in spleen size. He added that ascorbic acid improved iron absorption in the spleen. Heat stress decreased female GSI by about 88%($P<0.05$) and 12% in groups, non supplemented (B) and supplemented with AA (C, Table 1). This result may be attributed to the insufficiency of energy which is required for reproduction, due to loss of appetite during heat stress. This is almost similar to the finding of Spieler et. al., (1977). Ascorbic acid supplementation counteract the adverse effect of heat stress through its stimulative effect in boisynthesis of sex steroids. Levine and Morita, (1985) reported that ascorbic acid may act as a regulator in the biosynthesis of oestrogens in the follicular cells. Soliman et. al. (1986) found that ascorbic acid had an important role in maturation of the ovaries in *O. mossambicus*. The achieved changes in the present experiment for male GSI were insignificant ($P>0.05$).This result may be attributed to the limited temperature fluctuations. This result is in agreement with the finding of Madeline and Meier, (1983).

Finally, it could be easily concluded that ascorbic acid may play a physiological role in male reproduction i.e tests growth, however this needs further investigation.

5- Hematological response

During the first two weeks of high water temperature exposure hemoglobin and hematocrit did not significantly ($P>0.05$) affecting, thereafter, they were significantly increased in fish exposed to high water temperature. Heat stress increased hemoglobin concentration by about 22 % ($P<0.05$) and 50 % ($P<0.01$) and hematocrit by about 38% ($P<0.05$) and 63% ($P<0.01$) at the fourth and sixth weeks of experimental period, respectively (Figs.4 and 5). These results may be attributed to the stimulating effect of heat stress on red blood cells liberation from spleen and subsequently erythropoiesis (Tun and Houston, 1986). On the other hand, both of hemoglobin concentration and hematocrit value of group C exposed to the same high water temperature but fed ascorbic acid, were not significantly affected ($P>0.05$) by heat stress except at the sixth week, since they were significantly ($P<0.05$) lower in group C than in group A (Figs. 4 and 5). This finding is almost similar to that found by Dheer (1988).

6- Serum constituents

Serum glucose concentration in group B (exposed to heat stress) was twice as much as in group A (control) during the first four experimental weeks of heat exposure. Thereafter, it was lower in group B than in group A (Table 2 and Fig.6). The significant increase of serum glucose could be attributed to high cortisol secretion under the effect of heat stress (Abdel Hady, et.al. 1993 and Kobeisy et. al. 1997). However, the decrease of serum glucose at sixth week of the experiment may be related to the depletion of cortisol hormone and/or body energy stores as a result of prolonged heat stress. In contrast, group C did not significantly affect ($P>0.05$) due to heat stress. This result may be attributed to the physiological role of ascorbic acid on decreasing glucocorticoids synthesis by the adrenal cortex. These results are in harmony with those found by Thaxton and Pardue, (1984) and consequently gluconeogenesis.

Serum total protein concentration in group B (exposed to heat stress) tended to be lower than in group A (control). Such decrease was mainly due to the decrease of serum globulin rather than albumin concentration (Table 2). In fact high mortality rate in this group (B, Fig.2) may be attributed to the decrease of serum globulin as a result of lymphatic involution and suppression of immune system during heat

stress. This is in harmony with the results found by Shul 'man, (1974). Contrary, serum total protein concentration of group C (supplemented with AA) tended to be higher than in group A (control group). This increase was mainly due to the increase in serum albumin concentration (Table 2).

Serum cholesterol and triglycerides concentrations tended to be higher ($P<0.05$) in group B than in group A at the sixth week of the experiment (heat exposure). While, serum cholesterol and triglycerides concentrations were not significantly affected by ascorbic acid supplementation. However, ascorbic acid supplementation decreased serum cholesterol and triglycerides concentrations by about 14 and 17 %, respectively. Similarly, John et. al. (1979) found that circulating cholesterol levels were increased in ascorbic acid deficient fish. This result coincided with the increase of female GSI (Table 1), since the pronounced increase in cholesterol required for egg formation resulted in a significant decrease of serum cholesterol concentration (Waagbø et. al. 1989)

Heat stress increased significantly ($P<0.05$) the overall means of serum AST and ALT concentrations by about 58 and 28 %, respectively. While both enzymes did not significantly affect by heat stress with ascorbic acid supplementation (group C, Table 2) Similar trend was found by Shaffer, et. al. (1981) who attributed the high levels of AST and ALT enzymes may be related to the increase of their activities to accelerate the metabolic rate during heat stress. Similar observations were also found by Kobeisy et. al. (1997) due to heat stress.

Serum alkaline phosphatase concentration tended to be lower in fish exposed to heat stress, while ascorbic acid treated group had higher ($P<0.05$) concentration of serum alkaline phosphatase (Table 2). Similar results were found by some researchers such as Wilson (1973) and Lovell and Lim (1978) who found that the decrease of alkaline phosphatase activity coincided with the decrease of dietary ascorbic acid.

In conclusion, high water temperature (33 °C) had remarkable adverse effects on growth performance and some blood constituents of *Oreochromis niloticus*. The dietary ascorbic acid [3g/kg feed on dry matter (DM) basis] may be potent to counteract the adverse effects of high water temperature on growth performance, mortality rate and some blood constituents of *Oreochromis niloticus* under subtropical environmental conditions prevailing in Upper Egypt.

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Table 1: The influence of heat stress on average body weight gain and relative organ weights of *Oreochromis niloticus* fed ascorbic acid

Item	Experimental period (week)	Control (Group A)	Heat stress without ascorbic acid (Group B)	Heat stress with ascorbic acid (Group C)
Average body gain (g/fish)	2	1.15 ± 0.24 [a]	- 1.49 ± 0.20 [b]	0.78 ± 0.12 [a]
	4	1.37 ± 0.29 [a]	- 1.36 ± 0.27 [b]	1.60 ± 0.72 [a]
	6	1.80 ± 0.25 [a]	- 0.92 ± 0.16 [b]	1.50 ± 0.68 [a]
Overall mean		**1.44 ± 0.17**	**- 1.26 ± 0.15**	**1.29 ± 0.22**
2-Mean length increment (mm)	2	3.0 ± 0.04 [a]	- 1.5 ± 0.06 [b]	2.6 ± 0.02 [a]
	4	2.6 ± 0.05 [a]	0.4 ± 0.05 [b]	2.0 ± 0..04 [a b]
	6	2.8 ± 0.01 [a]	- 0.6 ± 0.04 [b]	1.9 ± 0.03 [a]
Overall mean		**2.8 ± 0.10**	**- 0.57 ± 0.17**	**2.17 ± 0.19**
Condition factor (K value)	2	1.85 ± 0.05 [a]	1.84 ± 0.05 [a]	1.82 ± 0.06 [a]
	4	1.87 ± 0.07 [a]	1.75 ± 0.06 [a]	1.77 ± 0.07 [a]
	6	1.87 ± 0.05 [a]	1.77 ± 0.07 [a]	1.88 ± 0.03 [a]
Overall mean		**1.86 ± 0.01**	**1.79 ± 0.02**	**1.82 ± 0.03**
Hepatosomatic index (HSI)	2	2.88 ± 0.28 [a]	2.36 ± 0.52 [a]	2.79 ± 0.53 [a]
	4	3.40 ± 0.66 [a]	3.00 ± 0.62 [a]	2.46 ± 0.25 [a]
	6	3.12 ± 0.24 [a]	2.55 ± 0.20 [b]	2.93 ± 0.36 [a]
Overall mean		**3.13 ± 0.13**	**2.64 ± 0.16**	**2.73 ± 0.12**
Spleensomatic index (SSI)	2	0.15 ± 0.01 [ab]	0.11± 0.03 [b]	0.17± 0.03 [a]
	4	0.13 ± 0.01 [a]	0.14 ± 0.01 [a]	0.18 ± 0.03 [a]
	6	0.16 ± 0.02 [ab]	0.13 ± 0.01 [b]	0.18 ± 0.01 [a]
Overall mean		**0.15 ± 0.01**	**0.13 ± 0.01**	**0.18 ± 0.00**
Female gonadosomatic index (fGSI)	2	2.09 ± 0.58 [a]	0.13 ± 0.01 [b]	1.66 ± 0.00 [a]
	4	1.32 ± 0.83 [a]	0.29 ± 0.01 [b]	1.35 ± 0.23 [a]
	6	1.75 ± 0.61 [a]	0.22 ± 0.02 [b]	1.52 ± 0.10 [a]
Overall mean		**1.72 ± 0.19**	**0.21 ± 0.04**	**1.51 ± 0.08**
Male gonadosomatic index (mGSI)	2	0.26 ± 0.09 [a]	0.31± 0.16 [a]	0.18± 0.04 [a]
	4	0.28 ± 0.13 [a]	0.14 ± 0.01 [a]	0.18 ± 0.04 [a]
	6	0.26 ± 0.11 [a]	0.26 ± 0.06 [a]	0.20 ± 0.02 [a]
Overall mean		**0.27 ± 0.01**	**0.24 ± 0.04**	**0.19 ± 0.01**

Means within rows differ ($P<0.05$) when superscripts differ.

Table 2: The effect of heat stress on some blood constituents of *Oreochromis niloticus* fed ascorbic acid

Item	Experimental period (week)	Control (Group A)	Heat stress without ascorbic acid (Group B)	Heat stress with ascorbic acid (Group C)
Glucose (mg/dl)	2	50.00 ± 6.34 ^{a}	94.87 ±30.82 ^{b}	47.44 ± 4.53 ^{a}
	4	54.70 ± 21.35 ^{a}	110.26 ±23.57 ^{b}	52.14 ± 7.73 ^{a}
	6	47.95 ± 0.00 ^{a}	41.54 ± 9.07 ^{b}	51.29 ± 10.88 ^{a}
Overall mean		**50.88 ± 1.73**	**82.22 ± 18.03**	**50.29 ± 1.25**
Total protein (g/dl)	2	4.02 ± 0.12 ^{a}	2.84 ± 0.49 ^{ab}	3.63 ± 0.30 ^{b}
	4	3.96 ± 1.46 ^{a}	3.39 ± 0.83 ^{a}	4.89 ± 0.91 ^{b}
	6	3.01 ± 0.00 ^{a}	2.92 ± 0.29 ^{ab}	4.39 ± 0.00 ^{b}
Overall mean		**3.66 ± 0.28**	**3.05 ± 0.15**	**4.30 ± 0.32**
Albumin (g/dl)	2	1.21 ± 0.12 ^{b}	1.77 ± 0.13 ^{ab}	2.39 ± 0.26 ^{a}
	4	2.49 ± 0.26 ^{a}	2.87 ± 0.22 ^{b}	3.30 ± 0.03 ^{a}
	6	1.20 ± 0.05 ^{b}	2.42 ± 0.54 ^{ab}	2.85 ± 0.32 ^{a}
Overall mean		**1.63 ± 0.37**	**2.35 ± 0.28**	**2.85 ± 0.23**
Globulin (g/dl)	2	2.81 ± 0.24 ^{a}	1.07 ± 0.36 ^{b}	1.24 ± 0.04 ^{ab}
	4	1.47 ± 0.31 ^{a}	0.52 ± 0.06 ^{b}	1.59 ± o.25 ^{a}
	6	1.81 ± 0.42 ^{a}	0.50 ± 0.04 ^{b}	1.54 ± 0.21 ^{a}
Overall mean		**2.03 ± 0.35**	**0.70 ± 0.16**	**1.46 ± 0.09**
A/G ratio	2	0.43 ± 0.04 ^{a}	1.65 ± 0.13 ^{a}	1.93 ± 0.21 ^{a}
	4	1.69 ± 0.11 ^{a}	5.52 ± 0.18 ^{b}	2.08 ± 0.07 ^{a}
	6	0.66 ± 0.08 ^{a}	4.84 ± 0.09 ^{b}	1.85 ± 0.06 ^{a}
Verall mean		**0.93 ± 0.34**	**4.00 ± 1.03**	**1.95 ± 0.06**
Total cholesterol (mg/dl)	2	161.66 ± 48.36 ^{a}	158.03 ± 1.10 ^{a}	170.99± 10.26 ^{a}
	4	122.28 ± 12.44 ^{a}	123.32 ± 7.33 ^{a}	146.12± 10.99 ^{a}
	6	145.47 ± 16.36 ^{a}	162.18 ± 8.79 ^{b}	125.56± 8.78 ^{a}
Overall mean		**143.14 ± 9.90**	**147.84 ± 10.67**	**147.56 ± 11.37**
Triglycerides (mg/dl)	2	99.53 ± 5.22 ^{a}	151.95 ± 3.52 ^{a}	95.66 ± 5.66 ^{a}
	4	92.80 ± 1.40 ^{a}	93.50 ± 2.14 ^{a}	95.13 ± 3.41 ^{a}
	6	110.80 ± 3.94 ^{a}	146.28 ± 6.91 ^{b}	92.12 ± 1.40 ^{a}
Overall mean		**101.04 ± 4.55**	**130.58 ± 16.12**	**94.30 ± 0.95**
AST (u / l)	2	44.00 ± 2.12 ^{a}	68.00 ± 7.84 ^{a}	41.00 ± 0.00 ^{a}
	4	44.33 ± 8.08 ^{a}	65.00 ± 8.25 ^{b}	33.50 ± 1.77 ^{a}
	6	38.00 ± 8.70 ^{a}	67.00 ± 5.55 ^{b}	37.25 ± 2.39 ^{a}
Overall mean		**42.11 ± 1.78**	**66.67 ± 0.76**	**37.25 ± 1.88**
ALT (u / l)	2	21.00 ± 0.00 ^{a}	29.50 ± 3.18 ^{a}	21.00 ± 0.00 ^{a}
	4	22.33 ± 0.89 ^{a}	29.00 ± 0.00 ^{b}	20.50 ± 1.01 ^{a}
	6	21.80 ± 1.15 ^{a}	25.00 ± 2.83 ^{b}	20.75 ± 1.18 ^{a}
Overall mean		**21.71 ± 0.33**	**27.83 ± 1.23**	**20.75 ± 0.13**
Alkaline phosphatase (IU / l)	2	23.44 ± 0.00 ^{a}	17.86 ± 1.36 ^{a}	24.36 ± 1.42 ^{a}
	4	23.92 ± 0.18 ^{a}	18.68 ± 0.98 ^{b}	27.22 ± 1.16 ^{a}
	6	24.54 ± 0.54 ^{a}	20.24 ± 1.28 ^{b}	25.14 ± 1.25 ^{a}
Overall mean		**23.97 ± 0.28**	**18.93 ± 0.60**	**25.57 ± 0.74**

Means within rows differ ($P<0.05$) when superscripts differ.

Fig. 1. Average body gain of O.niloticus exposed to heat stress (33°C) and fed ascorbic acid (AA).

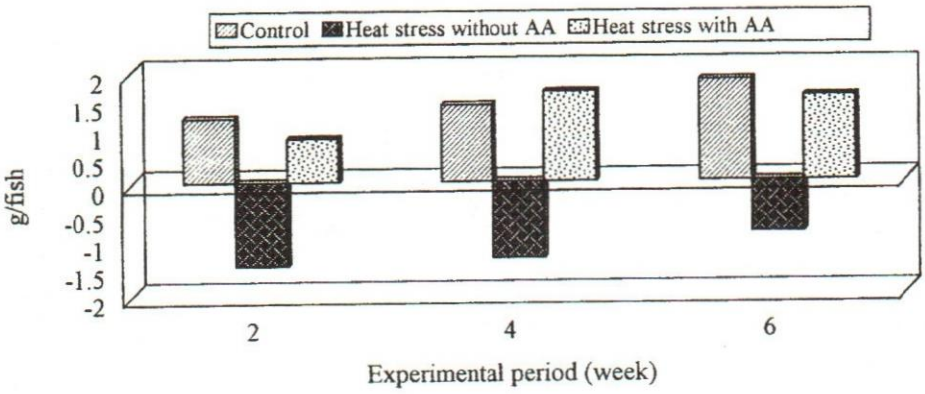

Fig. 2. Mortality rate of O.niloticus exposed to heat stress (33 °C) and fed ascorbic acid (AA).

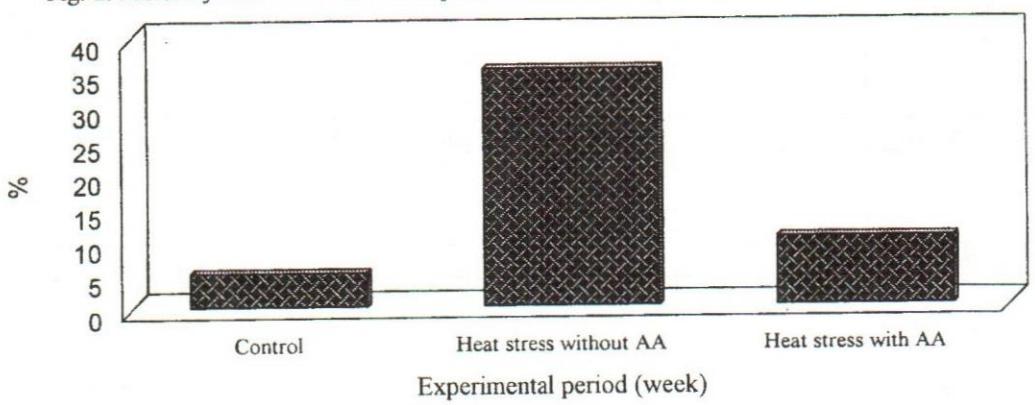

Fig. 3. Average spleensomatic index of O.niloticus exposed to heat stress (33 °C)and fed ascorbic acid (AA).

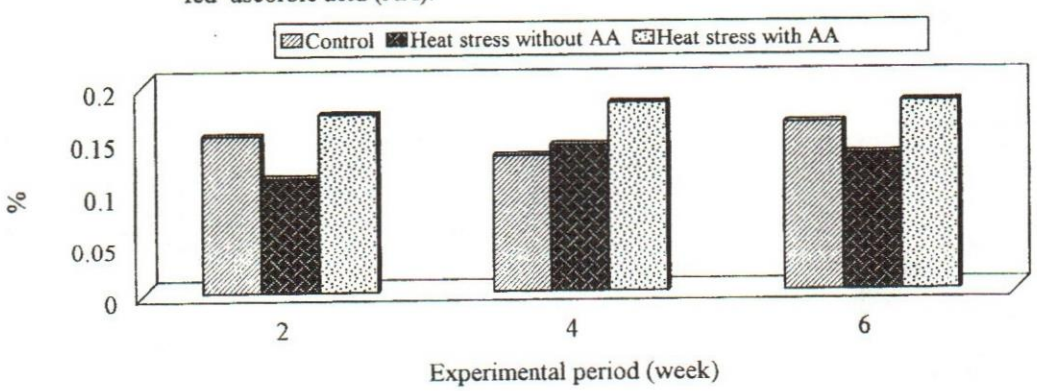

Fig. 4. Hemoglobin concentrations of O.niloticus exposed to heat stress (33 °C)and fed ascorbic acid (AA).

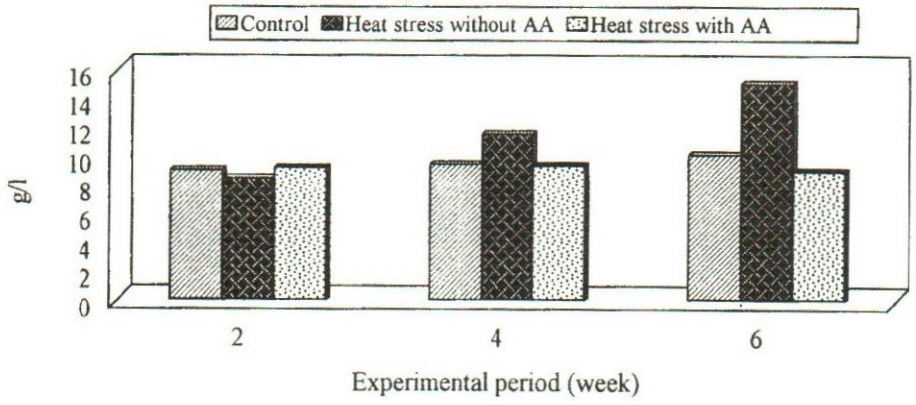

Fig. 5. Hematocrit values of O.niloticus exposed to heat stress (33 °C) and fed ascorbic acid (AA).

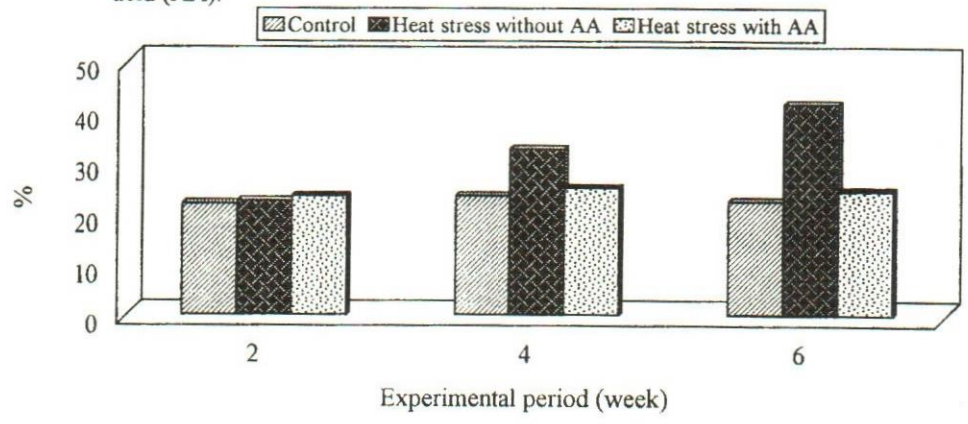

Fig. 6. Glucose levels of O.niloticus exposed to heat stress (33 °C)and fed ascorbic acid (AA).